Determination of selected pharmaceutical compounds in biosolids by supported liquid extraction and gas chromatography–tandem mass spectrometry☆

Beatriz Albero, Consuelo Sánchez-Brunete, Esther Miguel, Ramón Aznar, José L. Tadeo*

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A B S T R A C T

In this work, an analytical method was developed for the determination of pharmaceutical drugs in biosolids. Samples were extracted with an acidic mixture of water and acetone (1:2, v/v) and supported liquid extraction was used for the clean-up of extracts, eluting with ethyl acetate:methanol (90:10, v/v). The compounds were determined by gas chromatography–tandem mass spectrometry using matrix-match calibration after silylation to form their *t*-butyldimethylsilyl derivatives. This method presents various advantages, such as a fairly simple operation for the analysis of complex matrices, the use of inexpensive glassware and low solvent volumes. Satisfactory mean recoveries were obtained with the developed method ranging from 70 to 120% with relative standard deviations (RSDs) $\leq$ 13%, and limits of detection between 0.5 and 3.6 ng g^{-1}. The method was then successfully applied to biosolids samples collected in Madrid and Catalonia (Spain). Eleven of the sixteen target compounds were detected in the studied samples, at levels up to 1.1 μg g^{-1} (salicylic acid). Ibuprofen, caffeine, paracetamol and fenofibrate were detected in all of the samples analyzed.

1. Introduction

Pharmaceutical substances are a source of increasing environmental concern and together with endocrine disrupting chemicals have been classified as the most frequently detected organic contaminants in the environment [1]. The determination of pharmaceuticals in the environment has focused on the aquatic compartment because it has been reported that wastewater treatment plants (WWTPs), especially those with conventional technology, cannot accomplish the removal of these compounds before the effluents are discharged to surface waters [2]. Microbial degradation and the high tendency of some pharmaceuticals to remain adsorbed onto activated sludge are mechanisms that may explain the removal of pharmaceuticals during wastewater treatment [3]. Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge. When treated and processed, sewage sludge becomes biosolids, which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth. In the European Union, around 4 million metric tons (dry weight) of biosolids are annually applied to agricultural land [4]. Therefore, it is important to develop analytical methods for the detection of pharmaceuticals at trace levels to study their occurrence, behavior and fate in this complex matrix. The analysis of pharmaceutical drugs in sludge or biosolids has been focused on a limited set of compounds, mainly antibiotics [5–7] or non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. naproxen, diclofenac, ketoprofen, and ibuprofen) [3,8]; however, recent studies have included many more compounds belonging to several therapeutic classes that exhibit very different physicochemical properties [9–11].

However, the determination of these compounds in solid environmental samples is still scarcely documented, due primarily to a lack of appropriate analytical methods. Several methodologies have been developed for determination of pharmaceuticals in sewage sludge using pressurized liquid extraction [3,7,10–12], ultrasound assisted extraction [1,6,13], microwave assisted extraction [8] and QuEChERS [9].

Most published works regarding the analysis of pharmaceutical compounds in the environment were initially performed using mass spectrometry coupled to gas chromatography (GC–MS)

[8,14–17] or liquid chromatography (LC–MS) [18]. Nowadays, the majority of the analytical methods for the separation and detection of pharmaceuticals uses liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10,19–23]. Nevertheless, GC–MS/MS is an interesting alternative to LC due to its high resolution, lower operation costs and reduced solvent waste. Moreover, there is a clear impact on electrospray ionization of these compounds when working with complex matrices such as biosolids producing either ion enhancement or suppression that hinders the quantification of target compounds [1]. GC–MS/MS was used in the determination of pharmaceuticals in sewage water with very low detection limits [24]; however, its performance has not been evaluated for biosolid samples. Most pharmaceuticals possess functional groups with active hydrogen atoms (amines, amides, hydroxyl or phenolic groups) and a chemical derivatization of these groups, to reduce their polarity while increasing their thermal stability and volatility, is needed before their analysis by GC. Silylation with different reagents that lead to the formation of trimethylsilyl (TMS) or *t*-butyldimethylsilyl (*t*BDMS) derivatives is an effective approach for their determination.

Supported liquid extraction (SLE) is a relatively new technology that has been developed to replace classical liquid–liquid extraction (LLE). SLE involves the immobilization of aqueous samples over a solid inert phase, generally high purity diatomaceous earth that has a high capacity to retain water. The subsequent extraction is performed with any solvent that is immiscible with water. This technique presents several advantages over LLE, such as the reduced sample and solvent volumes required and no formation of emulsions [25]. Since emulsions are not an issue, solvent mixtures can be used regardless of density [26]. One advantage of SLE over solid-phase extraction (SPE) is that no preconditioning of the column is required; hence, the sample is directly loaded into the solid support, which shortens the whole extraction procedure. Unlike SPE, the whole sample is absorbed onto the solid support and there is no flow-through. This technique has been mainly applied with biological fluids such as plasma [25,27], although it has also been used in the determination of chemical warfare agents in water [28], polyphenols in wine [29] and pesticides in honey [30].

The aim of this work was to develop a rapid and sensitive multiresidue method based on SLE followed by GC–MS/MS for determination of 16 frequently used pharmaceuticals in biosolid samples. GC–MS/MS was selected to analyze a high number of compounds of different classes in a single run with a high selectivity. The selected pharmaceuticals present wide range of physico-chemical properties and belong to different therapeutic classes: NSAIDs, antiepileptics, antidepressants, lipid regulators, nervous stimulants and β-blockers. The simultaneous determination of pharmaceutical drugs with different physico-chemical characteristics requires a compromise in the selection of experimental conditions for all the compounds studied. A significant advantage of the described procedure is the reduction in the consumption of organic solvents and time versus previously published methodologies. To the best of our knowledge; this is the first time that SLE has been applied in the multiresidue determination of pharmaceutical drugs in biosolids.

2. Materials and methods

2.1. Sewage sludge collection

Pelletized biosolids from Madrid (Spain) were used in the development and optimization of the analytical method. Samples were put in amber glass jars (250–500 g) and stored at −20 °C until processing. The developed method was applied to the analysis of biosolids from WWTPs located in Madrid and Catalonia (Spain).

2.2. Reagents and standards

Methanol, ethyl acetate, acetone and acetonitrile (ACN), residue analysis grade, were purchased from Scharlab (Barcelona, Spain). A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to provide ultrapure water in this study. Formic acid was purchased from Sigma-Aldrich (St Louis, MO, USA). ISOLUTE SLE+ 1 mL supported liquid extraction columns were purchased from Biotage (Uppsala, Sweden).

N-*t*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA, purity > 97%), a mixture of MTBSTFA and *t*-butyldimethylchlorosilane (*t*BDMCS) (99:1, v/v), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, purity > 99%) and the mixture of BSTFA and trimethylchlorosilane (TMCS) (99:1, v/v), used as silylation reagents, were purchased from Aldrich (Steinheim, Germany). Pyridine was purchased from Panreac (Barcelona, Spain).

The standards of clofibric acid, ibuprofen, caffeine, salicylic acid, paracetamol, allopurinol, gemfibrozil, fenoprofen, amitriptyline, metoprolol, naproxen, mefenamic acid, ketoprofen, carbamazepine, diclofenac and fenofibrate were of analytical grade (purity >99%) and purchased from Sigma-Aldrich (St Louis, MO, USA).

Individual stock standard solutions were prepared in methanol at a concentration of 5 μg mL^{-1} and stored in amber vials at −18 °C in the dark. A working mixture solution containing 1000 ng mL^{-1} of all compounds was prepared in ACN weekly by dilution of the stock solution.

2.3. Sample preparation

An amount of 0.5 g pelletized biosolids, previously ground, was weighed into a 15 mL screw-cap glass tube and for the recovery assays, aliquots of the working mixture solution were added to reach final concentrations of 100, 50 and 25 ng g^{-1} allowing 24 h before extraction at 4 °C to reach equilibrium. After the addition of 2 mL acetone-1% aqueous formic acid (2:1, v/v), the mixture was stirred intensively by magnetic agitation for 60 min. Then, the extract was centrifuged at 4000 rpm for 4 min. A 500 μL aliquot of the supernatant was transferred to a glass tube and diluted (1:1, v/v) with 1% aqueous formic acid. The diluted sample was loaded on the SLE column and left 5 min for the sample being completely absorbed. Then, it was placed in a multiport vacuum manifold (SupelcoVisiprep, Madrid, Spain) and the analytes were eluted with 2 × 5 mL of ethyl acetate:methanol (90:10, v/v), applying vacuum for 5 min to complete elution. The extract was evaporated to dryness using a Genevac EZ-2 evaporator (NET Interlab, S.A.L., Spain) and the analytes reconstituted in 100 μL acetonitrile and pipetted into a 2 mL vial with a micro insert. The *t*BDMS derivatives were prepared by the addition of 50 μL of MTBSTFA:*t*BDMCS (99:1, v/v), then the vial was capped and placed in an oven at 70 °C for 1 h. After the derivatization step, the vial was left to cool down before performing the chromatographic analysis.

2.4. Gas chromatography–tandem mass spectrometry analysis

GC–MS/MS analysis was performed with an Agilent 7890A gas chromatograph equipped with a multimode inlet (MMI) and coupled to a triple quadrupole mass spectrometer, Model 7000 (Waldbronn, Germany). The MMI was operated in solvent-vent mode with gas liner with deactivated glass wool. During the 2 μL injection, at a rate of 40 μL min^{-1}, the split vent was open for 0.18 min with an inlet pressure of 5 psi and a flow rate of 100 mL min^{-1}. Once the entire sample has been injected, the inlet was switched to splitless mode for analyte transfer. After 2.68 min, the purge valve was activated at a 60 mL min^{-1} flow rate. The MMI program started at 60 °C kept for 0.18 min after injection,

Table 1
MS/MS parameters for the analysis of target pharmaceuticals in biosolids.

Compound	t_R (min)	Q^a	q^b
Clofibric acid	10.19	143 > 69 (5)	271 > 143 (5)
Ibuprofen	10.34	263 > 75 (15)	263 > 161 (20)
Caffeine	10.54	194 > 55 (30)	194 > 109 (15)
Salicylic acid	10.75	309 > 73 (20)	309 > 195 (15)
Allopurinol	11.16	307 > 193 (25)	307 > 166 (35)
Paracetamol	11.29	322 > 248 (30)	322 > 150 (35)
Gemfibrozil	11.64	243 > 83 (10)	243 > 73 (30)
Fenoprofen	11.83	299 > 75 (20)	300 > 75 (20)
Amitriptyline	11.91	202 > 200 (40)	202 > 201 (25)
Metoprolol	12.06	223 > 72 (10)	324 > 239 (10)
Naproxen	12.16	287 > 75 (25)	287 > 185 (25)
Mefenamic acid	12.59	298 > 224 (20)	224 > 180 (35)
Ketoprofen	12.61	311 > 75 (35)	311 > 295 (10)
Carbamazepine	12.84	193 > 165 (30)	193 > 167 (25)
Diclofenac	12.97	352 > 75 (25)	214 > 179 (25)
Fenofibrate	13.05	139 > 111 (15)	273 > 139 (15)

[a] Q: quantifier transition (collision energy, eV).
[b] q: qualifier transition (collision energy, eV).

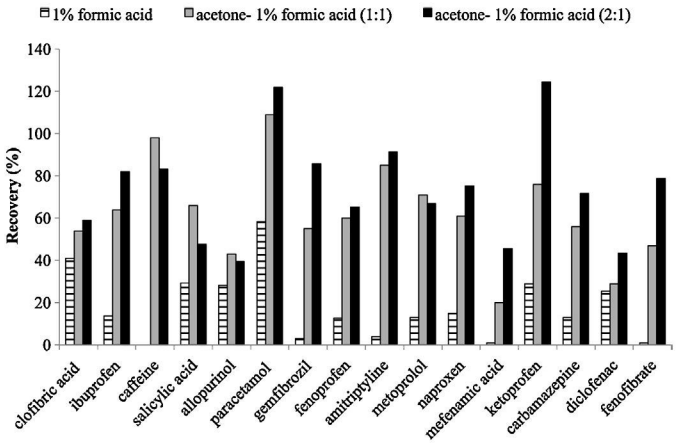

Fig. 1. Effect of the extraction solvent in the recovery of the studied pharmaceuticals.

then ramped to 325 °C at 600 °C min^{-1}, held 5 min, and finally decreased to the initial temperature cooling with compressed air. The chromatographic analysis was carried out using a fused silica capillary column ZB-5MS, 5% phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 μm film thickness) from Phenomenex (Torrance, CA). Helium (purity 99.995%) was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The oven was programmed to start at 60 °C (held for 3 min) and reach 300 °C at 20 °C min^{-1} where it was maintained 1.5 min. The total analysis time was 16.5 min and the run was carried out with a solvent delay of 10 min.

The mass spectra and the retention time of each of the analytes were acquired operating in full scan mode with a mass range from 50 to 540 *m/z*, scan time of 300 ms and an ion source and transfer line temperatures of 230 and 280 °C, respectively. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV. The electron multiplier was set with a gain factor of 6. Precursor ions for each analyte were selected taking into consideration a high ion *m/z*-value and abundance. The product ion spectra were obtained by the dissociation of the precursor ions at collision energies ranging from 5 to 50 eV. For quantitative analysis, multiple reaction monitoring (MRM) mode was employed with one quantifier and one qualifier transitions for each target compound. The dwell time in the different time segments was kept between 10 and 30 ms to achieve chromatographic peaks that gave good quantitative data. Analytes were confirmed by their retention time and the identification of target and qualifier transitions. Retention times must be within ±0.2 min of the expected time and qualifier-to-target ratios within a 20% range for positive confirmation. Table 1 summarizes the retention times and the transitions with their optimal collision energy used for quantification and confirmation of each analyte in MRM mode.

3. Results and discussion

3.1. Optimization of the sample preparation

Pharmaceuticals selected in this study present different physicochemical properties but most are acidic compounds, thus the extraction from biosolids (0.5 g) was carried out with 2 mL of acetone–0.5% aqueous formic acid (2:1, v/v). In a preliminary study, six pharmaceuticals (ibuprofen, salicylic acid, paracetamol, naproxen, ketoprofen and diclofenac) were extracted from samples spiked at 200 ng g^{-1} with the assistance of sonication (1 h) or magnetic stirring (1 h). Sonication produced lower extraction yields than those obtained with magnetic stirring (e.g. salicylic acid: 55% and 73%, respectively). Therefore, the extraction of pharmaceuticals from biosolids was performed with the assistance of magnetic stirring.

An aliquot of the centrifuged extract (0.5 mL) was diluted (1:1, v/v) with 1% aqueous formic acid before loading the SLE column to promote an even flow of the sample through the column, and analytes were eluted with 2 × 2.5 mL of ethyl acetate. In this first cleanup step, three different extraction mixtures (2 mL) were assayed: (a) 1% formic acid, (b) acetone–1% formic acid (1:1, v/v) and (c) acetone–1% formic acid (2:1, v/v). In general, higher extraction yields were obtained when a higher proportion of acetone was used (Fig. 1). In order to improve extraction yields, the elution from the SLE columns with 3 × 2.5 mL and 2 × 5 mL of ethyl acetate was assayed. An increase in the recovery was observed for salicylic acid (78%), fenoprofen (89%), gemfibrozil (95%) and diclofenac (78%) when higher elution volumes were applied, but the recoveries with ethyl acetate were low for pharmaceuticals such as clofibric acid, mefenamic acid, metoprolol and allopurinol. To improve the recovery of these compounds, the polarity of the extraction solvent was increased and a mixture of ethyl acetate:methanol (90:10, v/v) was tested. The elution with this mixture provided extraction yields >70% for all the pharmaceuticals in biosolid samples spiked at 100 ng g^{-1} (Table 2).

3.2. Derivatization

Silylation, commonly used for the derivatization of pharmaceuticals before their GC analysis, was employed in this study [24,31]. Two different silylation reactions were evaluated leading to the formation of TMS and *t*BDMS derivatives. Hence, BSTFA: pyridine (1:1, v/v), and BSTFA:TMCS (99:1, v/v) were tested to prepare the former and MTBSTFA: pyridine (1:1, v/v) and MTBSTFA: *t*BDMCS (99:1, v/v) to form the latter.

The derivatization conditions used for the silylation were based on those applied in our previous works where other phenolic compounds were derivatized [32,33]: 50 μL of the reagent were added to 100 μL of 5 μg mL^{-1} standard in ACN in a reaction vial that was capped and then heated at 70 °C for 30 min. The derivatized pharmaceuticals were then analyzed by GC–MS in the scan mode monitoring *m/z* from 50 to 540. Fig. 2 shows the peak areas obtained with the four different derivatization reagents. It was observed that pyridine was not as effective as the corresponding chlorosilane catalyst and lower peak areas were achieved when the silylating agent was mixed with pyridine. The preparation of *t*BDMS derivatives resulted in a significant increase in the response of several compounds, such as salicylic and clofibric acids, carbamazepine

Table 2
Average recovery[a] of pharmaceutical compounds from biosolids spiked ($n=4$) at three concentration levels, and limits of quantification (LOQ) and detection (LOD).

Compound	Recovery (%) 100 ng g^{-1}	50 ng g^{-1}	25 ng g^{-1}	LOD (ng g^{-1})	LOQ (ng g^{-1})
Clofibric acid	72 ± 4	76 ± 2	115 ± 11	3.1	10.3
Ibuprofen	95 ± 4	99 ± 2	102 ± 3	1.0	3.3
Caffeine	99 ± 11	103 ± 11	107 ± 3	1.7	5.5
Salicylic acid	89 ± 5	87 ± 6	115 ± 12	1.1	3.6
Allopurinol	79 ± 8	83 ± 9	100 ± 10	0.7	2.4
Paracetamol	92 ± 13	102 ± 10	117 ± 8	2.5	8.3
Gemfibrozil	70 ± 2	87 ± 11	120 ± 6	1.5	4.8
Fenoprofen	95 ± 8	97 ± 11	80 ± 5	3.1	10.4
Amitriptyline	107 ± 4	108 ± 5	98 ± 10	3.6	12.1
Metoprolol	84 ± 6	85 ± 7	106 ± 8	0.5	1.8
Naproxen	91 ± 6	86 ± 3	107 ± 9	0.8	2.6
Mefenamic acid	93 ± 13	73 ± 4	89 ± 3	1.6	5.2
Ketoprofen	80 ± 4	95 ± 8	114 ± 4	1.7	5.6
Carbamazepine	96 ± 9	109 ± 7	90 ± 5	1.5	5.0
Diclofenac	98 ± 7	94 ± 8	108 ± 7	0.7	2.3
Fenofibrate	90 ± 8	95 ± 4	96 ± 4	1.2	4.2

[a] Results are the mean (%) of four replicates ± RSD (%).

and allopurinol. In the case of paracetamol, the di-*t*BDMS derivative obtained presented a considerably higher peak area than the mono-*t*BDMS derivative. A 30 min derivatization step is not enough time for the complete reaction of two reactive hydrogen atoms of paracetamol. On the other hand, when BSTFA is used to derivatize paracetamol only one TMS moiety is attached but its chromatographic response is much lower than that obtained with the other reagent. As shown in Fig. 2, MTBSTFA–*t*BDMCS (99:1, v/v) was the reagent that produced, in general, the derivatives with higher chromatographic response and was chosen to enhance the volatility of the studied pharmaceuticals. Furthermore, it has been reported that *t*BDMS derivatives are about 1000–10,000 fold more stable to hydrolysis conditions than the TMS derivatives [34], which is an important feature taking into account that the analytes were extracted from biosolids using an aqueous solution.

The effect of derivatization time was studied at 30, 60 min and 24 h. After 30 min, the derivatization of paracetamol was not complete and a 60 min period was necessary to ensure the derivatization of all target compounds and the chromatographic response of the analytes after 24 h did not increase. The experimental conditions for the simultaneous derivatization of these compounds were set as follows: acetonitrile was selected as solvent for the silylation reaction and of 50 μL of MTBSTFA:*t*BDMCS, were added to a 100 μL sample and the mixture was kept at 70 °C during 60 min.

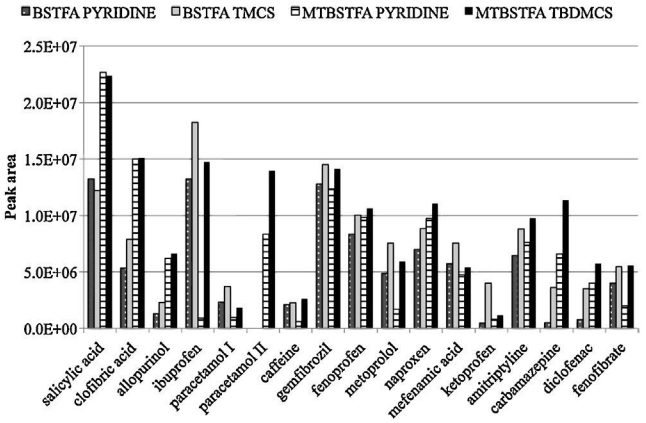

Fig. 2. Effect of the derivatization reagent on peak area of the 16 pharmaceuticals studied. Paracetamol I represents mono-*t*BDMS derivative and Paracetamol II is the di-*t*BDMS derivative.

3.3. GC–MS/MS optimization

Once the derivatization conditions were optimized and the formation of *t*BDMS derivatives was accomplished, the GC–MS/MS method was optimized. The most abundant ion observed in the mass spectra of the *t*BDMS derivatives was $[M-57]^+$ that results from the loss of the *t*-butyl moiety and was chosen as one of the precursor ions to evaluate possible product ions. Product ions of two different precursor ions were obtained performing the dissociation of the precursor ions at collision energies that ranged from 5 to 50 eV. Three of the studied pharmaceuticals, caffeine, fenofibrate and amitriptyline, do not have active hydrogen atoms so they do not undergo silylation as the rest of the studied pharmaceuticals. The fragmentation of amitriptyline using EI ionization produces a very poor spectrum and the most abundant ion is *m/z* 58, corresponding to the tertiary ammonium moiety, and ions above *m/z* 60 are of relatively low abundance. In the GC–MS analysis of amitriptyline, ions *m/z* 58 and 202 are usually monitored [35,36] and we selected the latter as precursor ion. Due to the low abundance of this ion, the resulting MRM used for quantification was the lowest of all the pharmaceuticals studied. To reduce the possible memory effects of the column, the inlet was flushed by heating at 300 °C for 30 min before the analysis of samples and procedural blanks were analyzed after every four samples.

The chromatographic response of target analytes may be affected by the presence of matrix components. Matrix effects were evaluated preparing two sets of standard solutions, one set of standards was solvent-based ranging from 10 to 1000 ng mL^{-1}, and the other was prepared spiking blank biosolid extracts in the same concentration range. For matrix-matched standards, the different concentration levels were prepared using the same volume of extract with the aim of maintaining the same amount of matrix throughout the range of concentrations studied. The slopes obtained by plotting peak area against seven concentration levels, following linear regression analysis, were compared. No significant matrix effects (<10%) were observed in seven of the analytes studied: clofibric acid, ibuprofen, salicylic acid, fenoprofen, naproxen, carbamazepine and fenofibrate. An enhancement of the chromatographic response due to matrix components was only observed in two compounds, allopurinol (13%) and diclofenac (74%). Although in gas chromatography matrix coextractives, in general, enhance analytes response, signal suppression was observed in the rest of the pharmaceuticals. The derivatization step is carried out with the reagent in excess, nevertheless, matrix components may compete with our analytes and thus a lower chromatographic response is observed in samples prepared spiking biosolid extracts. A decrease

Table 3
Levels of pharmaceuticals (ng g^{-1} d.w.) found in biosolid samples (n = 3).

Compound	BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10
Ibuprofen	95 ± 4	238 ± 19	21 ± 2	82 ± 4	350 ± 29	322 ± 26	444 ± 12	64 ± 3	344 ± 12	418 ± 14
Caffeine	74 ± 5	50 ± 6	153 ± 12	37 ± 2	86 ± 3	104 ± 6	63 ± 7	154 ± 3	132 ± 10	69 ± 4
Salicylic acid	825 ± 96	494 ± 55	126 ± 24	210 ± 10	414 ± 87	892 ± 88	651 ± 19	n.d.	751 ± 47	1111 ± 34
Paracetamol	192 ± 34	201 ± 27	24 ± 1	37 ± 5	119 ± 2	116 ± 14	161 ± 23	45 ± 7	134 ± 15	290 ± 37
Allopurinol	5.3 ± 0.1	6.4 ± 0.7	4.1 ± 0.8	3.6 ± 0.2	5.1 ± 0.5	7.8 ± 2.2	n.d.	n.d.	n.d.	7.5 ± 0.4
Gemfibrozil	57 ± 3	67 ± 3	n.d.	37 ± 2	61 ± 4	n.d.	n.d.	n.d.	n.d.	n.d.
Fenoprofen	58 ± 8	53 ± 2	n.d.	n.d.	n.d.	66.5 ± 9	50 ± 3	n.d.	70 ± 1	66 ± 12
Amitriptyline	177 ± 14	n.d.	n.d.	27 ± 3	n.d.	n.d.	n.d.	156 ± 10	n.d.	n.d.
Naproxen	n.d.[a]	n.d.	n.d.	n.d.	n.d.	23 ± 7	n.d.	n.d.	15 ± 2	12 ± 1
Diclofenac	278 ± 19	627 ± 69	n.d.	34 ± 4	97 ± 7	514 ± 56	180 ± 14	n.d.	258 ± 28	390 ± 35
Fenofibrate	302 ± 18	32 ± 1	13 ± 1	44 ± 4	135 ± 20	48 ± 4	47 ± 2	68 ± 1	101 ± 5	44 ± 3

[a] n.d.: not detected (<LOD).

of 28–37% in the chromatographic response was observed for gemfibrozil, paracetamol, amitriptyline and metoprolol, whereas for mefenamic acid and ketoprofen, the signal suppression was 76% and 86%, respectively.

3.4. Method validation

After optimization, the developed method was evaluated in terms of linearity, precision, accuracy and detection limits before it was used to determine pharmaceutical drug residues in biosolids. The validation procedure was carried out with matrix-matched calibration to counteract the observed matrix effect.

3.4.1. Linearity

Biosolid extracts spiked at seven concentration levels ranging from 5 to 500 ng g^{-1} were used to obtain the calibration curve of all the target analytes. Each calibration solution was injected three times, and a good linearity was obtained in the range assayed with correlation coefficients ≥0.990, except for paracetamol and diclofenac that was 0.982 for both.

3.4.2. Recovery

Recoveries were determined as the average of the analyses of four replicates of samples spiked at three concentration levels (25, 50 and 100 ng g^{-1}) by comparing these results with those obtained for biosolid extracts that were spiked after the sample preparation and prior to the derivatization step. Good recoveries, ranging from 70 to 120%, were obtained for the three spiking levels assayed (Table 2). At the lowest spiking level, the recoveries and standard deviations obtained were somewhat higher. The background levels of some target analytes could explain the higher uncertainty in the recoveries at this concentration level.

3.4.3. Repeatability

The repeatability of the chromatographic method was evaluated by performing the analysis of a derivatized biosolid extract spiked at 50 ng g^{-1}. The sample was injected 10 times and the relative standard deviations (RSD) obtained for peak areas ranged from 1.5 to 12%. The highest RSD value for peak areas was obtained for paracetamol. The reaction rate of amide groups with silyl reagents is lower than that of phenol groups and, thus, after the derivatization step is possible that both mono- and di-*t*BDMS paracetamol derivatives are formed, although during the chromatographic analysis the derivatization reaction is completed. The repeatability of the whole analytical procedure was determined by analyzing seven samples spiked at 50 ng g^{-1} within a given day and the RSD calculated for the studied compounds ranged from 1.5% to 11.0%.

3.4.4. Limits of detection (LOD) and quantification (LOQ)

Ten replicates of biosolid extracts spiked at 10 ng g^{-1} level were analyzed in order to determine LODs and LOQs of the developed method. The equation to calculate the LODs was the following: LOD = $t_{99} \times S$, where t_{99} is Students' t value appropriate for a 99% confidence level and $n - 1$ degrees of freedom and S is the standard deviation of the replicate analyses. The LOQ was calculated as 10 times the standard deviation of the results of the replicate analysis used to determine LODs. Low limits were obtained due to the high selectivity and sensitivity of GC–MS/MS, allowing the determination of these compounds at the trace levels found in biosolids. As shown in Table 2, LODs ranged from 0.5 to 3.6 ng g^{-1} and LOQs from 1.8 to 12.1 ng g^{-1}, being amitriptyline the compound with the highest limit, since it is the one that presents the quantifier MRM with lowest abundance. The LODs obtained with the proposed method are in the same range or lower than those reported by other authors in the analysis of pharmaceutical compounds in biosolids [1,8–10,13].

3.5. Analysis of biosolid samples

The optimized method was applied to the determination of the concentration of pharmaceuticals in ten biosolid samples collected in the areas of Madrid and Catalonia. The analysis of samples was conducted in triplicate and the measured concentration of the pharmaceuticals found and the corresponding standard deviations are summarized in Table 3. Eleven of the sixteen studied compounds were found and at least four pharmaceuticals were detected in all the samples analyzed. Fig. 3 shows the GC–MS/MS chromatograms of two biosolids analyzed.

In general, the highest concentrations of pharmaceutical residues correspond to NSAIDs, with salicylic acid found at levels up to 1111 ng g^{-1}, which are considerably higher than those reported by Martin et al. [13] in digested sludge. Three out of the seven NSAIDs monitored (ibuprofen, salicylic acid, and fenoprofen) were detected in all the samples analyzed. Some NSAIDs, particularly ibuprofen, ketoprofen, naproxen and diclofenac, are common target compounds in the analysis of pharmaceuticals in the environment. In our study, we did not find residues of ketoprofen in any of the samples analyzed and naproxen was only found in three samples at low concentrations, from 12 to 23 ng g^{-1}. Jelic et al. [37] performed the monitoring of pharmaceuticals in three conventional WWTPs over a period of two years and no residues of both NSAIDs were detected in any of the samples analyzed concluding that these compounds do not accumulate in sludge. Ketoprofen was not detected or quantified in sewage sludge from WWTPs in Athens (Greece) [1] and Seville (Spain) [13], respectively. Saleh et al. [3] analyzed four NSAIDs in sewage sludge from a Swedish WTTP, and the highest concentration levels were found for ibuprofen in the range of 304–588 ng g^{-1} and the lowest concentration levels were for naproxen in the range 7 to 14 ng g^{-1}. It should be noted that these result are similar to those obtained in this work. Sagrista et al. [15] reported data on the occurrence of NSAIDs in sludge from Swedish STPs and they show concentrations about 29, 39, 122

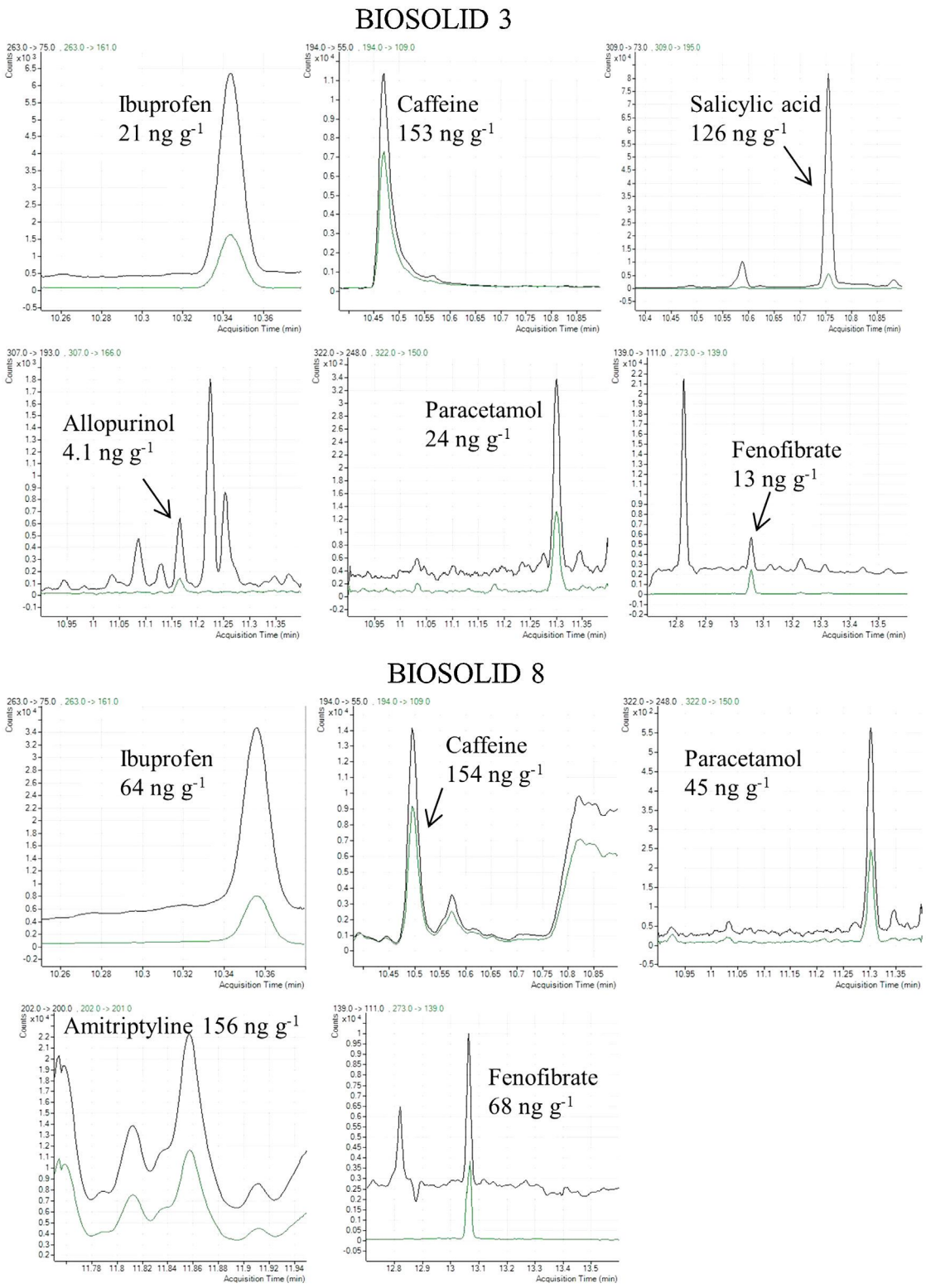

Fig. 3. GC–MS/MS chromatograms of the pharmaceuticals found in two of the biosolids samples analyzed.

and 138 ng g^{-1} for ketoprofen, diclofenac, ibuprofen and naproxen, respectively. Differences of these values with those obtained in this work could be attributed to the treatment processes performed in the WWTP or their concentration in inflow waters according to their use by consumers.

The anti-epileptic drug carbamazepine was not found in any of the samples at levels above LOD. Carbamazepine has a very low removal regardless of the treatment applied and does not adsorb onto the sludge [37,38] although it has been found in sludge from different locations [10,39,40].

The presence of caffeine in sludge is not only due to its therapeutic application as nervous stimulant, diuretic or to enhance the effect of analgesics but mainly because it is a constituent of a variety of beverages (coffee, tea, soft drinks) and food products

[41]. Caffeine was detected in all of the samples analyzed in the present study at levels that ranged from 37 to 154 ng g^{-1}, higher than the concentrations found by Nieto et al. [39], up to 74 ng g^{-1}, and by Okuda et al. [42], 16 ng g^{-1}. Allopurinol, drug used primarily to treat hyperuricemia, was the pharmaceutical compound found at the lowest levels with average concentrations in a range from 3.6 to 7.8 ng g^{-1}. To the best of our knowledge, this is the first time this compound has been determined in biosolids.

4. Conclusions

In this work, a method that combines SLE and GC–MS/MS was developed for the analysis of 16 pharmaceuticals in biosolids. Due to the reduced amount of sorbent used in the SLE procedure, only small quantities of both samples and organic solvents were required that resulted in a considerable saving of analysis time. An important advantage of this method is the ease of use and the wide range of compounds that could be extracted efficiently. Another significant advantage of the described procedure is the reduction in the consumption of organic solvents versus previously published methodologies.

Satisfactory results were obtained for all the compounds studied in terms of reproducibility (RSD ≤ 13%) and sensitivity with LODs ranging from 0.5 to 3.6 ng g^{-1}. The proposed method was applied to biosolids from two Spanish areas, Madrid and Catalonia, and at least 4 of the 16 target pharmaceuticals were detected in all the samples analyzed.

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